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The Effects of Estrogenic and Gonadotrophic Hormones on Normal Levels of Some Blood Chemical Constituents in the Non-Breeding Female Mallard Duck

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THE EFFECTS OF ESTROGENIC AND GONADOTROPHIC HORMONES ON NORMAL LEVELS OF SOME BLOOD CHEMICAL CONSTITUENTS IN THE NON-BREEDING FEMALE MALLARD DUCK

by

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INTRODUCTION

In past years, a substantial amount of research has centered on the levels of certain blood chemical constituents in birds, particularly in chickens and pigeons. However, little has been recorded concerning the blood chemical constituents of the mallard duck, Anas platyrhynchos. Likewise, the effects of hormones on its blood chemistry, in conjunction with its reproductive system, have been almost totally unexplored. Therefore, this study, conducted from October 14, 1965, to March 3, 1966, had as its purpose the determination of normal quantitative levels of certain blood chemical constituents (the electrolytes--calcium, sodium, potassium; the proteins--albumin and globulin; and lipids) and the measurement of the effects of estrogenic and gonadotrophic hormones on these constituents; and hence, on the reproductive system of the non-laying female mallard. Financial support for the work was supplied through a grant from The United States Department of Interior, Bureau of Sport Fisheries and Wildlife Service, contract number 14-16-0008-728.

Like most wild birds, the female mallard passes through distinct phases of reproduction alternating between a period of sexual rest (non-breeding condition) and one of sexual activity (breeding condition). Normally, the female mallard is able to reproduce only within the span of a few weeks during the spring of the year. The gonadal and other changes associated with re-

productive activity are controlled and regulated largely by endogenous hormones which are triggered in response to external stimuli (Witschi, 1959). In this study, it was hoped that the regulation of stimuli and the administration of hormones during the non-breeding period would serve to make conditions conducive to bringing about morphological and blood chemical changes similar to those occurring in the breeding period.

Some of the morphological changes at the onset of the breeding period in the reproductive system of fowl are brought about by gonadotrophic hormones. According to Witschi (1935), gonadal growth and ovulation are controlled by these hormones which originate in the pituitary gland or hypophysis. The gonadotrophins in fowl are termed Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). Though each of these has functions similar to its mammalian analogue, it is still not clear as to whether mammalian gonadotrophins are homologous to their avian counterparts. In fact, most work with mammalian gonadotrophins, where pigeons and chickens were used, has yielded inconclusive and often conflicting results. Chu and You (1946) were successful in stimulating follicular growth in pigeons when they used mammalian FSH; but, Nalbandov (1953) had only partial success with chickens. Other researchers found avian gonadotrophins, in the form of a chicken pituitary powder, able to elicit precocious follicular development where mammalian gonadotrophins failed (Das and Nalbandov, 1935). In this study, both mammalian

and avian gonadotrophins were utilized in an attempt to stimulate ovarian development, but primary attention was given to their influence on blood parameters.

Though it is sometimes doubted that gonadotrophins exhibit any direct effects on blood chemistry, it is commonly accepted that FSH does stimulate ovarian follicular growth, thus making probable an increased titer of the gonadal hormones, particularly estrogen, which have been found to have a definite effect on some areas of avian metabolism (Nalbandov, 1953; Lorenz, 1954; Urist, 1959; and Sturkie, 1965). Moreover, estrogen treatment in birds is known to cause an enlargement of the oviduct and its ligaments; and in addition to its morphological effects, estrogen causes marked changes in blood composition. As a basis for this statement, much research concerning the effects of estrogen (notably that of van Tienhoven, 1961) can be cited. Only that which is pertinent to this study will be discussed in the following documentation.

A noteworthy elevation of serum calcium and lipid has been observed to occur at the onset of laying in chickens (Polin and Sturkie, 1957; and Urist, 1959). Similarly, many investigators treating fowl of both sexes and various ages with estrogens have also observed significant increases in blood calcium and lipid (Landauer, et al., 1941; Mc Donald, et al., 1945; Fleischmann and Fried, 1945; Common, et al., 1948; and Urist, 1959). In both cases, this calcium elevation is agreed by most to be caused

by estrogen in the presence of another hormone from the parathyroid gland. This was demonstrated in the duck by French workers. They observed in parathyroidectomized ducks, that calcium does not elevate in response to estrogen as it does in intact ducks (Benoit, et al., 1941). Thus, the hormone from the parathyroid gland (parathormone) is believed to regulate the diffusive (unbound) blood calcium fractions, whereas estrogen augments the non-diffusive (bound) fractions. Polin and Sturkie (1957) and Urist (1960) confirmed this in the chicken. Urist found that when the parathormone maintained diffusive levels of calcium above 4 milligrams percent, estrogen was able to elicit an elevation of the non-diffusive fraction of serum calcium. However, when diffusive levels of calcium were below 4 milligrams percent, estrogen had little or no effect on the non-diffusive fraction. Consequently, the parathyroid hormone and its effects on serum calcium metabolism, though not dealt with in this study, can not be entirely ignored.

As previously mentioned, total serum lipids, as well as calcium, show an elevation in response either to estrogen treatment or to the onset of laying. Ramney and Chackoff (1951) have considered the liver to be the principal organ concerned with lipid metabolism in birds. Sizable daily dosages of estrogens have been reported to induce a pronounced blood lipemia as well as increased liver fat (Urist, 1959; Stamler, et al., 1950).

In addition to the effects estrogen has on calcium and lipid, marked changes have also been observed in serum protein. Urist (1959) demonstrated nearly a twofold increase in serum protein following estrogen treatment of chickens. He found the increase was totally accounted for in the globulin fraction while albumin showed a slight decrease.

Little has been reported regarding the effects of estrogen on serum sodium and potassium. Sodium is the primary electrolyte in serum and it is in much greater concentration than potassium. Both electrolytes are believed to be present in serum in the ionic form (Sturkie, 1965). Most measurements of serum sodium and potassium in fowl have been made without regard to physiological state. Moreover, many fluctuations in sodium and potassium have been linked to stress and the consequential release of adrenal steroids. Although, as yet, the specific adrenal steroids associated with physiological changes accompanying stress in birds has not been well defined, the possibility of stress and the role of adrenal steroids in serum sodium and serum potassium metabolism can not be overlooked.

METHODS AND MATERIALS

Source of Ducks

The ducks used in this study were mature semidomesticated and wild female mallards, Anas platyrhychos. The majority of the ducks were purchased from a commercial breeder--Whistling Wings, Hanover, Illinois. The remainder, which included nine males, were procured from the Kellogg Bird Sanctuary, Battle Creek, Michigan. All birds were banded for easy identification, and their flight feathers were clipped.

Housing and Feeding

A reserve flock of ducks was maintained on property in Portage, Michigan. The flock was composed of 40 to 70 female mallards and 9 male mallards. These birds were maintained in an enclosure of approximately 250 feet by 100 feet. A pond which measured 30 feet by 50 feet and which was lined with a heavy-duty polyethelene plastic, was located in the center of this area. The water depth varied from 0 to 2 feet (Figure 1). A small barn and an attached shed were accessible to the ducks. Inside the shed, there was a ground level watering trough which was heated with an electric immersion heater to keep the water from freezing during the winter months (Figure 2).

Figure 1: View of the pond on the Portage, Michigan, Property.

Figure 2: View of the shed which was accessible to the ducks.

Those ducks chosen for a particular hormonal experiment were selected from the reserve flock and transported in wooden crates to the live animal room in Wood Hall, Western Michigan University; Kalamazoo, Michigan. During the first two investigations, one to three ducks were housed per cage, depending upon the size of the cage. All cages were situated over drop pans which contained a fecal absorbant material. The drop pans were cleaned twice a week. The ducks were fed and watered from porcelain pans twice daily.

During the last two investigations, the ducks were housed one per cage, the cages measuring 24 by 36 by 28 inches. The floor of each cage was fitted with half-inch hardware cloth which gave better footing to the ducks and allowed fecal material to drop into water-filled drop pans located beneath each cage. Each cage contained a large watering trough ($6\frac{1}{2}$ by $22\frac{1}{2}$ by 5 inches), and a glass finger bowl for food (Figure 3). The ducks were fed daily and the drop pans and cages were hosed clean every other day.

Both the farm ducks and the experimental ducks were fed corn and wheat during the early part of the study. Later, a Napiana duck developer pellet was substituted for the wheat (for content, see Appendix A).

Blood Sampling Technique

The blood sampling technique used was a modification of

Figure 3: View of duck cages in the live animal room, Wood Hall, Western Michigan University.

the procedure employed by Decker (1965). The duck was grasped at the base of the wings, the head was tucked under a wing, and the anterior end of the duck was wrapped in a muslin bag. The duck was then placed on its back in the wooden blood sampling tray (Figures 4 and 5). An elastic immobilizing strap was secured over the duck to minimize movement during the blood sampling.

Because of its size and easy accessibility, the metatarsal vein, on the inside of either leg, was used to obtain all blood samples. An alcohol solution, applied with cotton, served as a cleanser and disinfectant. Pressure was applied to the metatarsal vein proximally to the knee joint. Then, a heparinized hypodermic needle was inserted into the metatarsal vein distally to the knee joint and immediately withdrawn. The blood was allowed to flow out of the wound and into a plastic ampule temporarily mounted to the blood sampling tray (Figure 5). Approximately 2 milliliters of blood were collected. If necessary, the metatarsal vein was clamped with the thumb and forefinger distally to the puncture to stop the bleeding, and a coagulant (Gelfoam powder, Upjohn Company) was applied to the wound.

The blood sample was allowed to clot under mild refrigeration for approximately 10 minutes. The clot was loosened and the blood sample was centrifuged at 3000 r.p.m. for 5 minutes in a small clinical centrifuge. Immediately following this, the supernatant serum was removed with clean disposable pipettes and transferred to clean glass test tubes. The serum samples were

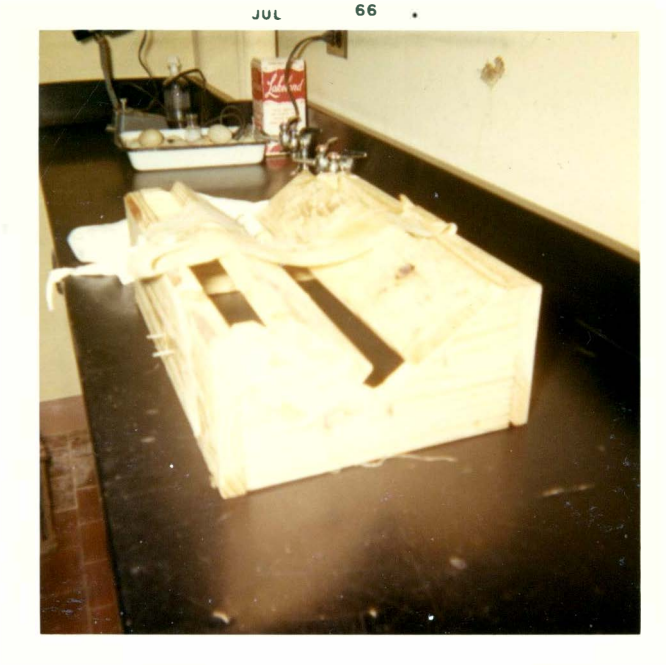

Figure 4: Blood sampling tray.

Figure 5: Obtaining a blood sample from a female mallard.

stored at -15° C until the time of analysis.

Analysis of Serum Samples

Blood serum samples were tested for quantitative levels of three cations (calcium, potassium, and sodium), protein, and lipids by the following procedures.

Cation analysis

Quantitative measures of the three blood serum cations were determined on a Hitachi Perkins-Elmer model 139 UV-VIS Spectrophotometer with a flame photometry attachment (139-0400) using hydrogen gas as the fuel.

Dilution curves were established with a control serum, Lab-trol (Dade Reagents Company), which also served as a standard. Serum specimens were diluted by 9:1 with a diluent consisting of 8 parts distilled water and 1 part 0.02 percent Sterox solution (Hartman Leddon Company). All diluted samples were placed in a shaking machine for five minutes prior to flame analysis. Finally, they were tested at the wave lengths listed below.

Ca^{++}	K^{+}	Na^{+}
422.7	404.6	589.6

Protein analysis

Using the Baush and Lomb Spectronic 20 Colorimeter, blood serum samples were tested quantitatively for albumin by the biuret

test as outlined in the Baush and Lomb Clinical Method for the Spectronic 20 Manual. Lab-trol was employed as a standard.

Selected blood samples were analysed electrophoretically for albumin and globulin by the method of Briere and Mull (1964). Total protein was determined by an American Optical T.S. meter. Serum protein fractions, albumin and globulin, were separated for thirty-five minutes on a cellulose acetate strip in a Gelman cell with a constant voltage of 300 volts. A Barbitone-Barbitol buffer solution was used (pH, 8.16; ionic strength, .005). Following this, the cellulose acetate strips were stained with Ponceau S stain, cleared with 5 percent acetic acid solution and placed in a Beckman Spinco analytical scanner to obtain measurements.

Lipid analysis

Quantitative tests for lipids were made by the procedure of Natelson (1961), using half the stated amounts of reagents and serum samples. Briefly, the procedure involved dissolving the selected lipid samples in chloroform, precipitating the protein with sulfuric acid, centrifuging, and removing, drying, and weighing the chloroform layer.

Experimental Methods

In the following recount of experimental methods, the farm control ducks, having been observed for the duration of the

study, are discussed apart from those ducks involved in the hormonal experiments. All of the latter, which were randomly selected and separated from the farm control birds, received hormone treatment by injection into the axillary space under either wing. They were housed in the laboratory as previously described. Methods used with the experimental birds are discussed in the order of the four experiments in which they were employed.

Farm control birds

Beginning October 14, 1965, ten female mallards were selected from the farm flock and sprayed with a green flurescent paint to distinguish them from the reserve flock. These marked birds will hereafter be referred to as the farm control birds.

Every Thursday, from October 14, 1965, to January 27, 1966, the entire flock was driven into a trap box (Decker, 1965) and the ten farm control ducks were removed. Blood samples were taken on the site and the birds were released until the following Thursday. The farm control ducks served to establish non-laying levels of the blood chemical constituents considered in this study and provided a basis for determining the influence of experimental procedure.

LH experiment

Ten female mallards were obtained from the reserve flock on November 15, 1965. They were weighed and divided into five groups of two. The birds were placed in a room with a temperature of approximately 24° C and with an 11 hour light--13 hour dark artificial illumination sequence which corresponded to the natural photoperiod at that time. They were housed and fed as previously described.

Over a period of ten days, three pairs of ducks (group 2, 3, and 4) were daily (11:00 A.M.) administered Armour Standard dosages of Pituitary Luteinizing Hormone (PLH) of equine origin, supplied by the Armour Baldwin Laboratories, Omaha, Nebraska. Group two was given 0.5 milligrams; group three, 1.0 milligrams; and group four 2.5 milligrams. The lyophilized PLH was reconstituted with .9 percent saline prior to injection and stored in a frozen state when not in use.

A chicken pituitary extract, CPX, prepared daily from five whole chicken pituitaries, was divided equally and administered (11:00 A.M.) for ten days to the birds of group 5. The chicken pituitaries were collected from both sexes, various ages and many breeds of chickens soon after they were killed in a nearby poultry slaughter house. In order to preserve potency, the pituitaries were immediately packed in dry ice, and later transferred to a freezer until they could be homogenized in 0.9 percent saline just prior to administration.

The remaining pair of ducks, group one, served as controls and were administered 0.9 percent saline daily for the ten day period.

Blood samples were obtained from each duck on each of two days prior to the hormonal administration period, every other day during that period (2,4,6,8,10), and on days 2 and 4 after that period. Blood samples were taken between 2:00 and 4:00 P.M. on the above days and analysed as previously described. At the conclusion of the experiment, all ducks were weighed and killed and their reproductive organs were examined.

E-estrogen experiment

Eight female mallards were utilized for this experiment, which was conducted from December 2 to December 18, 1965. They were weighed and separated into groups of two (groups E-1 through E-4). They were maintained in a room with a photoperiod of 11 hours light--13 hours dark, and with a temperature of approximately 18° C. They were housed and fed as previously described. Each group was treated with a different dosage of 17-β- estradiol supplied by the Upjohn Company of Kalamazoo, Michigan. The estradiol was dissolved in peanut oil and administered daily at 11:00 A.M. for a period of 14 days, according to the following schedule.

Group number	Dosage/day
E-1	25 micrograms
E-2	250 micrograms
E-3	2.5 milligrams
E-4	5 milligrams

Blood samples were obtained in the afternoons of three days prior to the hormone treatment period, and on days 1, 3, 5, 8, 11 and 14 during the hormone administration period.

At the conclusion of the study, one duck from group E-2, and one duck from group E-3 were killed and their ovaries and oviducts were removed and preserved.

R-estrogen experiment

In order to validate the findings of the E-estrogen experiment, the R-estrogen experiment was conducted January 4 to January 20, 1966, under similar photoperiod and temperature conditions. Ten female mallards were selected from the reserve flock, weighed and designated in pairs as groups R-1 through R-5. Each group was daily administered a different dosage of 17-β- estradiol, according to the following chart for 14 days.

Group number	Dosage/day
1	60 micrograms
2	125 micrograms

Group number	Dosage/day
3	250 micrograms
4	500 micrograms
5	1000 micrograms

Hormones were administered in the morning (11:00 A.M.), and blood samples were taken from each bird 3 days prior to the hormone treatment period and on days 1, 3, 7, 10 and 13 in the afternoon during the period of hormone administration. A final blood sample was obtained by cardiac puncture when the ducks were killed the day following the conclusion of the hormone treatments. Ovaries and oviducts were removed and preserved.

S-CPX experiment

This investigation was begun on February 9, 1966, and was conducted over a period of 23 days. On day one, 12 female mallards were weighed and assigned code numbers S-1 through S-12, placed in a room with a photoperiod of 12 hours light--12 hours dark, and with a temperature range of 19° C to 25° C. Ducks S-1 through S-8 and duck S-11 were administered a definite number of whole chicken pituitaries collected on two occasions from a nearby slaughter house. These were administered in the form of a chicken pituitary extract prepared as in the LH experiment. The ducks were divided into the groups

shown in the chart below and administered the CPX at 11:00 A.M. on days 6, 9, 17 and 20. Ducks S-9 and S-10 were control ducks and they received 0.9 percent saline on the same days.

Group	Bird number	Pituitaries
1	S-1, S-2	1
2	S-3, S-4	3
3	S-5, S-6	2
4	S-7, S-8, S-11	5
5	S-9, S-10	Control

Over a period of ten days, duck S-12 was daily treated with 0.1 milligrams of "purified" ovine follicle stimulating hormone, supplied by the National Institutes of Health, NIH-FSH-S3 (see Appendix B for assay).

Blood samples were taken from all ducks on days 1, 4, 7, 10, 13, 16, 19, and 23 of the experiment. Eggs were removed from the cages and recorded for day and bird.

Three days after completion of the experiment, ducks S-6 and S-12 were laparotomized. All of the remaining ducks were weighed and returned to the reserve flock.

Statistical Analysis

All data, tested for statistical significance, were processed in an IBM 1620 Central Processing Unit located in the

Computer Center at Western Michigan University. Significant difference between the means was the *t* test employed for all samples. A one-tailed test was used for calcium, albumin and globulin and a two-tailed test was employed for potassium. Serum sodium and lipid were not tested for statistical significance because of the large individual variations of the former and the small sample sizes of the latter. Results of the *t* tests are presented in Appendix H.

RESULTS

Normal Levels

Quantitative levels of serum calcium, sodium and potassium in the non-breeding mallard female were established from 156 blood samples obtained from the ten farm control ducks over a period of four months (October through January). Mean values, standard deviation and range are presented for each electrolyte in Table 1. To illustrate individual variation in serum electrolytes (calcium, sodium, and potassium) mean values were tabulated for each farm control duck. The ranges of mean values of serum calcium (10.3-11.1 milligrams percent), sodium (322-336 milligrams percent), and potassium (18.4-20.1 milligrams percent) show large differences between ducks in mean serum sodium values but relatively meager variations in mean serum calcium and potassium values (Table 2).

Large weekly fluctuations in serum sodium were recorded for many of the individual farm control birds, but no definite trend or pattern of change developed in serum sodium that could be directly attributed to the time of the experiment (October through January). Throughout the experiment, serum calcium and serum potassium levels remained relatively stable for all farm control birds (Appendix C). To illustrate weekly electrolyte fluctuations, graphs of the changes in two farm control ducks,

Table 1: Mean serum calcium, sodium, potassium, albumin and lipid for the non-breeding female mallard duck.

	Calcium (mg%)	Sodium (mg%)	Potassium (mg%)	Albumin (gm%)	Lipid (mg%)
Mean	10.8	331	19.3	3.42	316
Standard deviation	±.9	±17.9	±1.5	±.54	±44.7
Range	8.5-13.8	278-420	16.6-25.7	2.43-5.56	100-630

n = 156 for calcium, sodium, and potassium
n = 127 for albumin
n = 29 for lipid

Table 2: Mean values of serum constituents calcium, sodium, potassium and albumin for each of the ten non-breeding female mallard ducks.

Duck number	Calcium* (mg%)	Sodium* (mg%)	Potassium* (mg%)	Albumin+ (gm%)
1	11.0	329	19.6	3.36++
2	10.9**	335	19.4	3.21
3	10.3	328	18.8**	3.48++
4	10.6	331	19.6	3.40+++
5	10.5***	333**	19.2**	3.31++
6	10.4**	322**	18.4**	3.50++
7	10.9	333	18.4	3.43++
8	10.9	336***	19.9**	3.82
9	11.0	334	20.1	3.55
10	11.1	324	18.9	3.19

* n = 16 except for ** n = 15 and *** n = 14
+ n = 12 except for ++ n = 13 and +++ n = 14

numbers 1 and 7, are presented in Figures 6, 7, and 8.

Smoes (personal communication), in a study of female mallards housed under laboratory conditions, established laying levels of the same serum constituents considered in this study. He found mean calcium at 20.2 milligrams percent, sodium at 314 milligrams percent, and potassium at 16.6 milligrams percent. After comparing Smoes's values with those determined in this study, it became apparent that calcium underwent almost a twofold increase during the transition from non-breeding to breeding state. Other workers investigating the chicken found a corresponding elevation in serum calcium accompanying the laying state (Schjeide and Urist, 1956; Winget and Smith, 1959). Most researchers agree that endogenous estrogens from the ovary are responsible for this calcium elevation. In addition, the serum calcium values of 9.9-10.4 milligrams percent established by Landauer and coworkers (1941) for the mallard drake are very similar to those found in the non-breeding female mallard used in this study. Both sodium and potassium appeared slightly depressed during the laying state as opposed to the non-laying state in the female mallard.

Mean serum albumin, as analysed by the biuret test, was determined from 127 blood samples collected from the farm control birds over the duration of the study. This value, along with standard deviation and range are presented in Table 1. Mean serum albumin values for each duck can be found in Table 2.

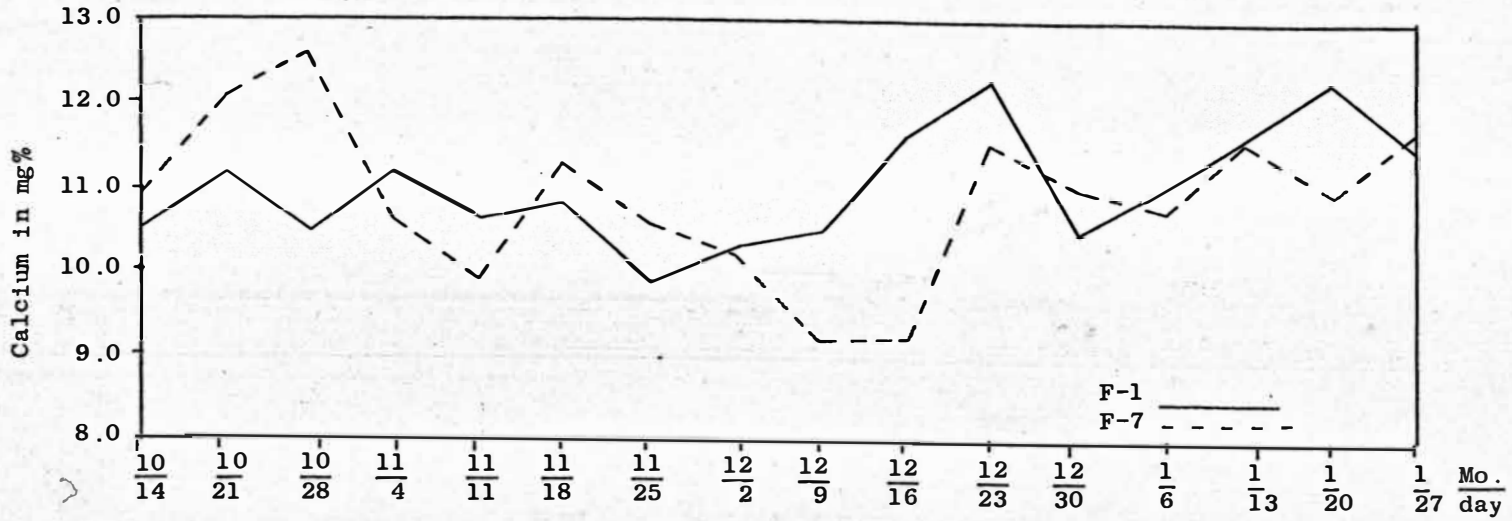

Figure 6: Weekly fluctuations in serum calcium for farm control duck 1 (F-1). and farm control duck 7 (F-7).

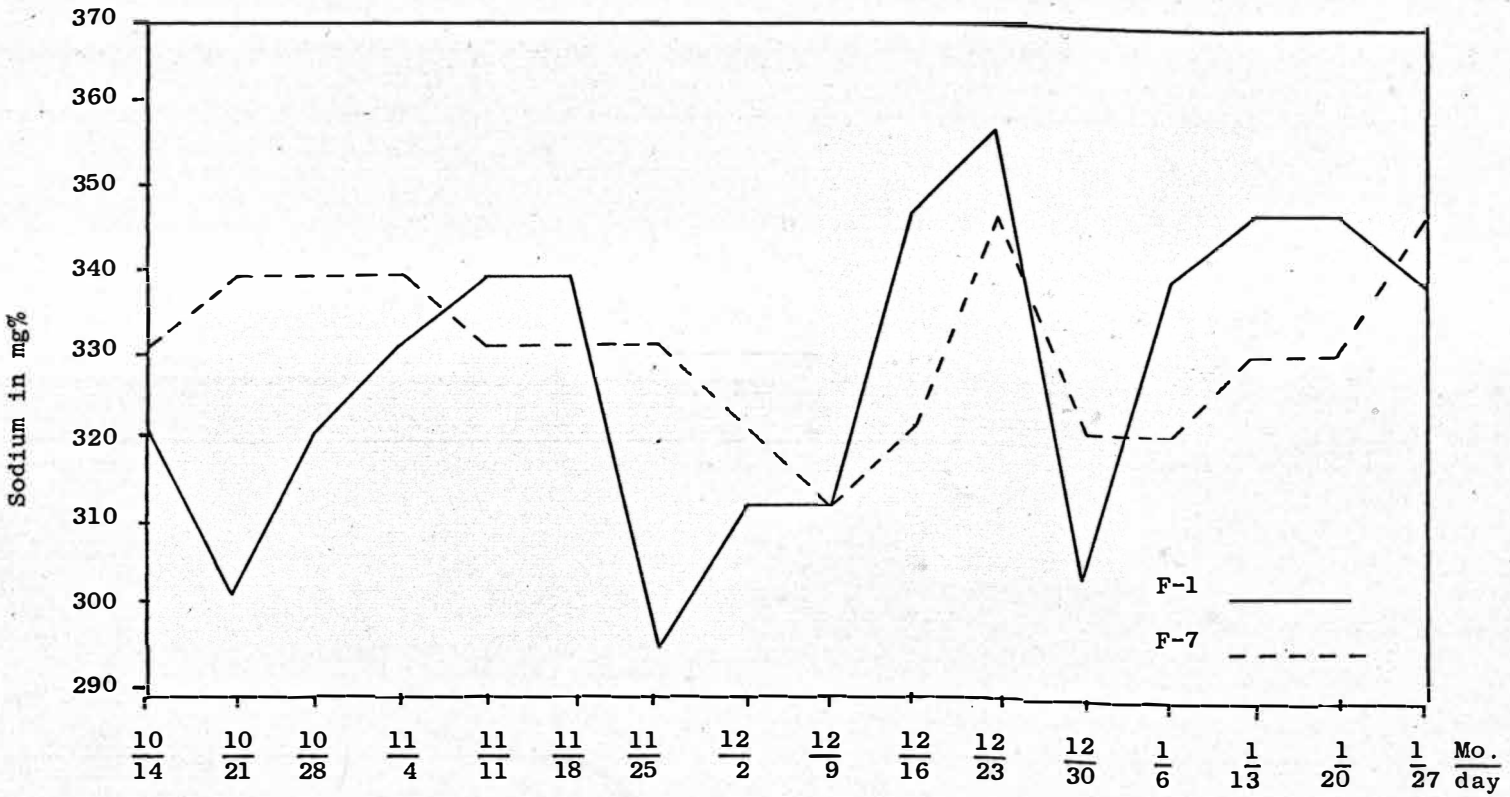

Figure 7: Weekly fluctuations in serum sodium for farm control duck 1 (F-1) and farm control duck 7 (F-7).

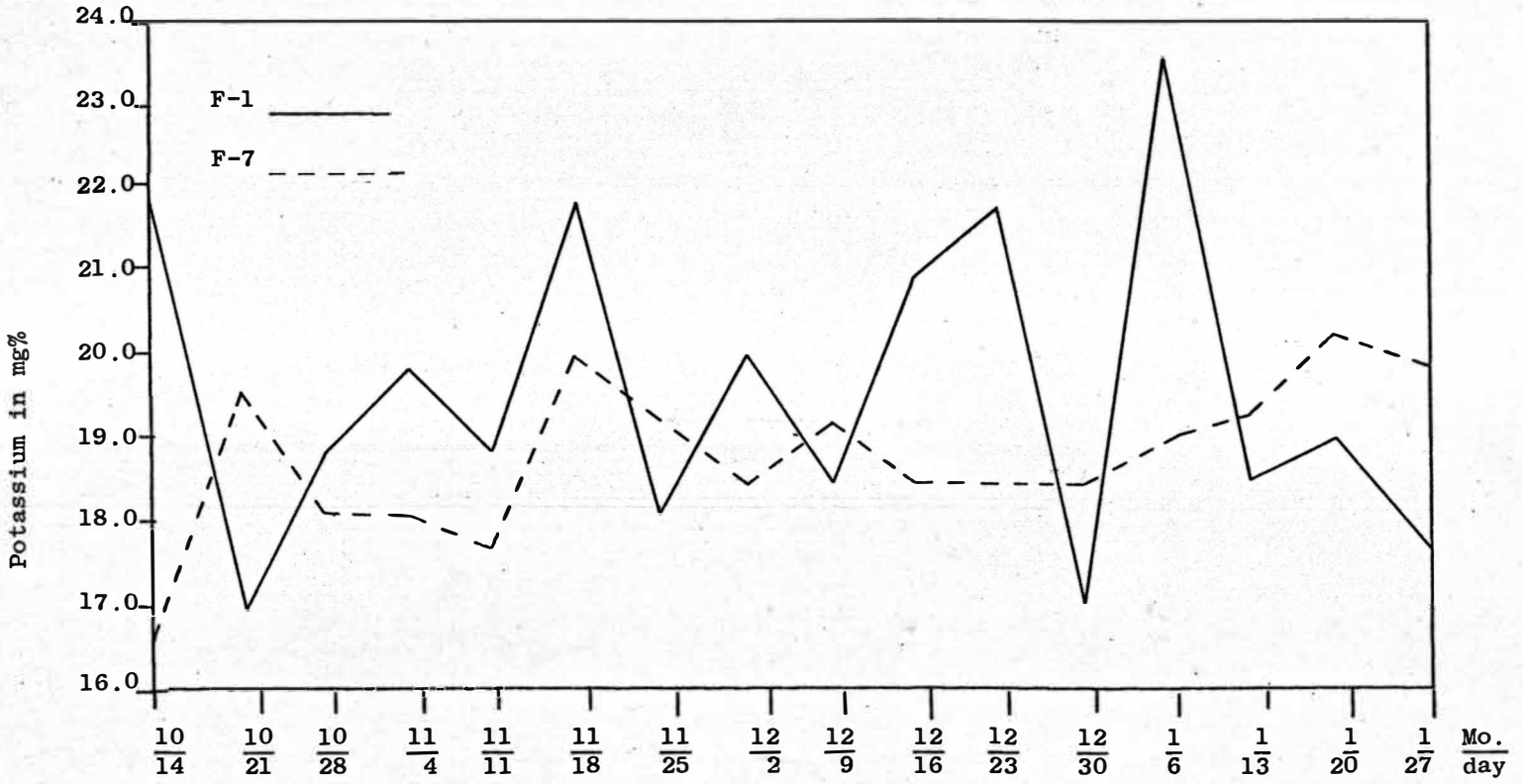

Figure 8: Weekly fluctuations in serum potassium for farm control duck 1 (F-1) and farm control duck 7 (F-7).

weekly albumin changes in the ducks used previously for Figures 6, 7, and 8 are presented graphically in Figure 9. Raw data on a weekly basis is presented for each farm control bird in Appendix C.

Thirty-nine selected serum samples from the farm control birds, already having been tested for albumin by the biuret method, were also analysed for globulin and albumin by electrophoresis. Mean values for these serum constitutents, along with serum total protein and albumin to globulin ratios (A/G) appear in Table 3.

Smoes (personal communication) measured 5.6 milligrams percent mean total protein, composed of 2.1 milligrams percent albumin and 3.5 milligrams percent globulin in the laying female mallard. From his results, it is evident that total protein increased during the laying state. The primary increase appeared in the globulin fraction, whereas albumin showed a slight decrease. This gave an overall lower A/G ratio during the breeding state. Brandt and colleagues (1951) also observed laying chickens to have a significantly larger globulin fraction in their blood serum than those not laying. Likewise, Urist (1959) found an increase in serum globulin in chickens following estrogen treatment. Therefore, the difference between the serum globulin fraction of the non-laying hen and the laying bird could be due to higher circulating levels of estrogen in the latter.

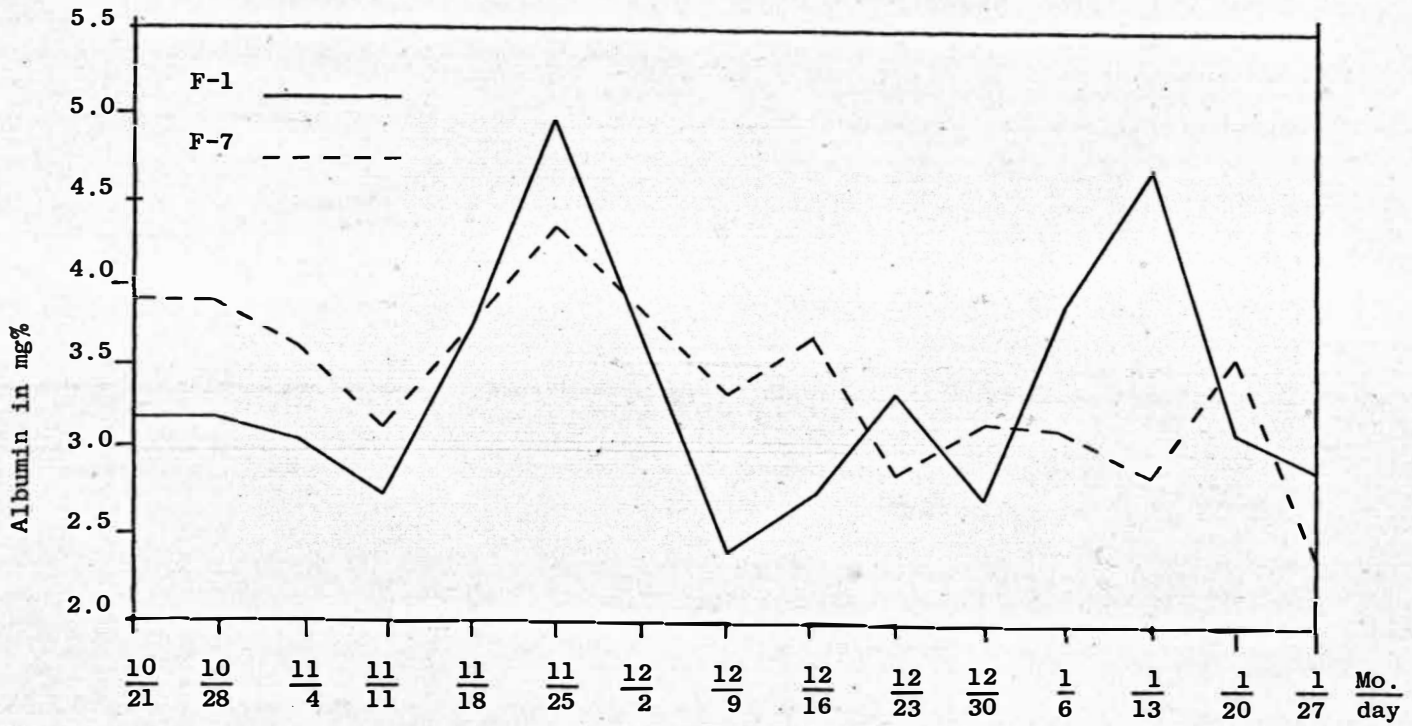

Figure 9: Weekly fluctuations in serum albumin for farm control duck 1 (F-1) and farm control duck 7 (F-7).

Table 3: Mean values for total serum protein, albumin, and globulin, and albumin to globulin ratio (A/G) for the non-breeding female mallard duck.

	Total protein (gm%)	Albumin (gm%)	Globulin (gm%)	A/G ratio
Mean	4.8	3.2	1.6	2.0
Standard deviation	±.58	±.51	±.41	
Range	3.8-6.1	2.3-4.0	0.9-2.3	

n = 39

Mean serum lipid was established from 29 blood samples procured from the farm control birds over the duration of the study (Table 1). Large standard deviation and range (Table 1) and raw data (Appendix C) show that total serum lipid values varied appreciably in different ducks and at different times. These marked changes in serum lipid values over the duration of the study could have been related to changes in nutritional state (Sturkie, 1965).

Reproductive state, as well as nutrition, has been known to effect serum lipid concentration. Smoes (personal communication) found mean serum lipids in the laying female to be 850 milligrams percent, almost three times the values established for the non-laying mallard hen in this study. In agreement with this, research with chickens has shown that the rise in total plasma lipid with the onset of sexual maturity is a response to increased secretion of estrogen by the maturing ovary (Sturkie, 1965).

E-estrogen Experiment

In the E-estrogen experiment, pretreatment control levels of the serum constituents, calcium, sodium, potassium and albumin were tabulated for each treatment group from serum specimens obtained three days prior to and the first day of estradiol treatment. In addition, control values for these constituents were also tabulated from blood samples obtained from the farm control ducks. Treatment values of the above serum components

were calculated for each group from blood samples taken during the estradiol treatment period (Table 4). Weights before and after the experiment and the levels of the serum constituents are presented for each duck in Appendix D.

All mean treatment levels of serum calcium, except those for the ducks on 25 micrograms estradiol, showed a significant increase at the .01 level over mean pretreatment values (Table 4). This calcium elevation was so great at higher dosage levels that values exceeding 26.0 milligrams percent had to be estimated from an interpolation of the dilution curve. Landauer and coworkers (1941), treating male mallards, also observed a correlation between increased estradiol dosage and calcium elevation. However, in another experiment with male mallards they noted that though calcium again more than doubled following estradiol treatment, the elevation did not correspond directly to the dosage levels as it had in the previous experiment.

During the estradiol treatment, mean serum sodium showed no significant change from pretreatment values. However, large differences in serum sodium were often noted when blood samples were taken from individual ducks (Appendix D). In addition, large variations in mean serum sodium values were found for the different treatment groups and for individual ducks. Similarly, large variation between and within ducks in serum sodium was noted for the farm control birds. This variation can not be explained without more intensive study of serum sodium metabolism

Table 4: Farm control, pretreatment (P) and treatment (T) levels of serum calcium, sodium, potassium, and albumin in the female mallard duck on different daily dosage levels of estradiol.

Group	Estradiol dosage	Number of samples		Mean Calcium (mg%)	Mean Sodium (mg%)	Mean Potassium (mg%)	Mean Albumin (gm%)
Farm control	--	20		10.1	318	19.8+	3.2
1	25ug	4	P	10.9*	336	17.7	3.9
		10	T	10.6	334	19.1	3.7
2	250ug	4	P	9.6**	323	17.9	4.3
		9	T	15.8	325	18.7	3.7
3	2.5mg	4	P	10.5***	321	18.1	4.0
		10	T	23.3	323	20.1	5.3
4	5.0mg	4	P	10.4****	307	16.8	4.2
		10	T	26.1	308	18.2	4.9

* $\underline{t}$ = .61, df = 12
** $\underline{t}$ = -4.98, df = 11
*** $\underline{t}$ = -6.34, df = 12
**** $\underline{t}$ = -6.90, df = 12

\+ Potassium statistical analysis grand mean pretreatment/ farm control, $\underline{t}$ = 4.41, df = 34

in birds.

Serum potassium did demonstrate a slight increase over pretreatment levels. However, mean pretreatment values of serum potassium tabulated from ducks comprising each treatment group were significantly different (.05 level) than those recorded for the farm control birds at the time of this study. This difference could possibly be explained by the differences in environmental conditions in which the two flocks were maintained. A stress factor might also have been involved. Selye (1950), while relating research of others, points out that marked changes in serum potassium and sodium in mammals are associated with systemic stress. Since little research has dealt with birds, valid comparisons are difficult.

Serum albumin was slightly lower during treatment than before in groups 1 and 2; yet, the reverse was true for groups 3 and 4 (Table 4). Blood samples from the ducks in groups 2 and 3 were further analysed electrophoretically for albumin and globulin and for total protein on three days prior (pretreatment) and during the fifth day of the hormonal treatment period. Mean values for these treatment groups were tabulated and are presented in Table 5. As is evident from these figures, both groups of ducks showed a decrease in the A/G ratio during treatment even though total serum protein in the birds comprising group 2 was not appreciably increased. This was accounted for by an increase in the globulin fraction, and a corresponding decrease

Table 5: Mean pretreatment (P) and treatment levels of total serum protein albumin, globulin and albumin to globulin ratio (A/G) for two groups of female mallard ducks receiving different dosages of estradiol.

Group	Estradiol dosage	Number of samples		Total Protein (gm%)	Albumin (gm%)	Globulin (gm%)	A/G ratio
2	250ug	2	P	5.5	3.5	2.0	1.7
		2	T	5.7	3.0	2.7	1.1
3	2.5mg	2	P	4.8	2.8	2.0	1.4
		2	T	7.7	2.6	5.1	.51

in the albumin fraction. Urist (1959) reported like findings in chickens treated with estradiol. In the ducks receiving 2.5 milligrams estradiol daily, pretreatment and treatment levels of albumin were almost identical (Table 5). However, a large increase in the globulin fraction would seem to explain the large increase in total protein during treatment.

Frequently, in this and in other hormone experiments, electrophoretic and biuret tested albumin from the same serum specimens did not yield the same quantitative results. This was also found by Sturkie (1965). Electrophoretically tested serums often showed high globulin levels, especially in the estrogen experiments, while serums from the same blood samples tested by the biuret method showed abnormally high albumin measurements. The biuret technique may have produced these incongruous results by incompletely separating the globulin from the albumin fractions.

Total serum lipid values were determined for a bird from group 3 and for a bird from group 4, on three days prior to estradiol treatment and on the eighth day of estradiol treatment. The bird receiving 2.5 milligrams estradiol daily (from group 3) showed an elevation of serum lipid from 333 milligrams percent to 2500 milligrams percent; whereas the duck receiving 5 milligrams estradiol (from group 4) showed an increase from 425 to 3700 milligrams percent. Landauer and coworker (1941) observed almost a threefold increase in serum lipids in the

mallard drake following a daily 1 milligram estradiol treatment.

As noted by Lorenz and Bachman (1947), estrogens differ in their lipogenic potencies and they can cause fatty deposition in the liver and other organs. The estradiol used in this experiment seemed to be very lipogenic in the mallard hen, particularly at higher dosage levels. This was evidenced by a clearly visible lipid fraction in the serum which became more pronounced as estradiol treatment continued. In addition, fatty deposition of the liver was clearly apparent in a bird from group 3, and it was also evident, though to a lesser degree, in a duck from group 2.

The ovaries in both of the above mentioned birds appeared to be suppressed, while the oviduct of the bird from group 3 (2.5 milligrams estradiol) appeared well developed. The oviduct of the bird from group 2 appeared not to be stimulated. Indeed, this seemed to indicate that the levels of estrogen required to bring about morphological changes (i.e., oviduct enlargement) were much greater than those necessary to bring about changes in blood constituents (rise in calcium, protein, etc.) at that time of year, in as much as both birds demonstrated dramatic changes in blood chemistry, but only the bird on the higher dosage showed oviduct development.

R-estrogen Experiment

In the R-estrogen experiment, mean pretreatment levels of calcium, sodium, potassium, and albumin were determined for each treatment group from serum samples collected prior to estrogen treatment. Also, mean farm control serum values taken at the time of this phase of the study (January 6 and 13) were tabulated. Mean treatment values for the above serum constituents were tabulated from blood samples obtained during the time of estradiol treatment (Table 6).

In this experiment, as in the E-estrogen experiment, mean treatment values of serum calcium showed significant increase at the .01 level over mean pretreatment values at each dosage level of estradiol, though the increase was not linear with respect to dosage (Table 6). Landauer and colleagues (1941) reported similar results in an experiment with mallard drakes.

The ducks of the R-estrogen experiment seemed to be more responsive to estrogen treatment, as evidenced by a greater increase in serum calcium and by increased oviduct development at the lower dosage levels. Similarly, in the R-estrogen experiment, some pretreatment levels of calcium were higher than those found in the farm control birds at the time. These values were also higher than those observed in the E-estrogen experiment (Tables 4 and 6). The difference in time between the estrogen experiments--the R-estrogen study having been

Table 6: Farm control, pretreatment (P) and treatment (T) levels of serum calcium sodium, potassium and albumin in the female mallard duck treated with different daily dosage levels of estradiol.

Group	Estradiol dosage (ug)	Number of samples		Mean Calcium (mg%)	Mean Sodium (mg%)	Mean Potassium+ (mg%)	Mean Albumin (gm%)
Farm control	--	20		10.9	329	20.6	3.6
1	60	4	P	12.5*	348	19.7	3.2
		10	T	19.3	334	18.1	3.7
2	125	4	P	11.4**	350	20.5	3.0
		10	T	28.2	333	18.2	4.4
3	250	3	P	14.6***	343	18.4	3.8
		9	T	25.1	337	16.9	4.7
4	500	4	P	12.1****	351	17.0	3.5
		10	T	31.1	331	19.5	5.0
5	1000	4	P	10.9*****	346	17.1	2.9
		10	T	35.4	320	22.0	5.5

* t = -3.76, df = 12
** t = -15.18, df = 12
*** t = -2.80, df = 10
**** t = -7.96, df = 12
***** t = -8.68, df = 12
+ Potassium statistical analysis, grand mean pretreatment/grand mean treatment, t = -1.19, df = 67

conducted one month nearer the breeding period than the E-estrogen study--was probably related to the change in concentrations of serum blood constituents, and also to the changes in effects of estrogen on these constituents. This becomes even more apparent when the overall results of the two estrogen experiments are compared.

Serum sodium, at all treatment levels, particularly at the higher dosage levels (500-1000 micrograms) showed a decrease in response to estradiol administration. In contrast, in the E-estrogen experiment, there was a small increase in serum sodium during estradiol treatment at all dosage levels, except at 25 micrograms. However, in the R-estrogen experiment, pretreatment levels of sodium were determined from blood samples procured soon after the ducks had been changed from an outdoor to an indoor environment. This change in physical environment may have caused an elevation of serum sodium during the adjustment phase; for after a period, levels during treatment again approached the values established for the farm control birds at that time. Yet, extreme variability in serum sodium made almost any explanation questionable and statistical analysis misleading.

Serum potassium was higher during pretreatment than during estradiol treatment at the lower dosage levels (60, 125, and 250 micrograms), but, the reverse was true at the higher dosage levels (Table 6).

As dosage levels of estradiol increased, serum albumin increased linearly (analysis by the biuret technique). However, these values conflicted with those obtained by electrophoretic analysis, and it was believed that the biuret test was not removing all of the globulin fraction. Electrophoretic patterns showed a depression of albumin and an increase in globulin as treatment continued. Mean serum albumin and globulin values were tabulated for different dosage levels of estradiol on a pretreatment and again on a treatment basis. Pretreatment serum total protein, albumin and globulin were calculated from blood samples obtained three days before and the first day of estradiol treatment. Treatment levels of the above serum constituents were determined from blood samples obtained on days 10 and 14 during the estradiol treatment period (Table 7). The female mallard showed an increase in total protein at all dosage levels except at 250 micrograms. Moreover, the increase in total protein is accounted for in the globulin fraction, whereas albumin decreased. This was also true at the dosage level of 250 micrograms, where total protein decreased. The globulin increase and the albumin decrease during hormonal administration gave all treatment groups a lower A/G ratio which was consistent with both the E-estrogen experiment and with the results of previously cited literature.

In contrast to the E-estrogen experiment, serum lipid values determined for individual ducks in this study did not show any

Table 7: Mean pretreatment (P) and treatment (T) levels of total protein, albumin, globulin and albumin to globulin ratio (A/G) for female mallard ducks receiving different dosages of estradiol.

Group	Estradiol dosage (ug)		Total Protein (gm%)	Albumin (gm%)	Globulin (gm%)	A/G ratio
1	60	P	5.3	3.4	2.0	1.7
		T	5.9	2.0	3.9	.50
2	125	P	4.9	2.9	2.0	1.5
		T	6.4	2.1	4.3	.49
3	250	P	5.8	3.2	2.6	1.2
		T	5.6	2.2	3.4	.66
4	500	P	6.4	3.9	2.6	1.5
		T	7.2	2.1	5.1	.43
5	1000	P	4.7	3.0	1.7	1.7
		T	6.6	2.4	4.2	.58

n = 4

Albumin statistical analysis, grand mean pretreatment/grand mean treatment, $t = 4.18$, df = 38

Globulin statistical analysis, grand mean pretreatment/grand mean treatment, $t = -5.21$, df = 38

appreciable changes in response to estradiol treatment. However, pretreatment values appeared somewhat higher than those observed in the E-estrogen experiment. Some difficulty was encountered in obtaining a sufficient quantity (0.5 ml) of blood serum, so that many lipid values were calculated from serum samples smaller than the amount required by the procedure. This may have introduced considerable error into the determination of serum lipids in this experiment. Lipid values for each bird can be found in Appendix E.

At dosage levels of 500 micrograms and 1000 micrograms estradiol daily, the ovaries appeared very much suppressed, in comparison to those of other non-breeding ducks, whereas at lower dosage levels, follicles of considerably larger size were observed (Figure 10). However, estrogen dosage levels of 250, 500, and 1000 micrograms seemed to produce greater oviduct development than did smaller dosages. Since the administration of estrogen usually depresses the output of pituitary gonadotrophins FSH and LH in fowl (Nalbandov and Baum, 1948), it is not surprising to see little follicular development at higher dosage levels. At the same time, since estrogen is known to greatly increase the size of the oviduct in fowl and since increased oviduct weight in the chicken is commonly used as an assay for estrogen (Lorenz, et al., 1962), neither is it surprising to find an enlarged oviduct.

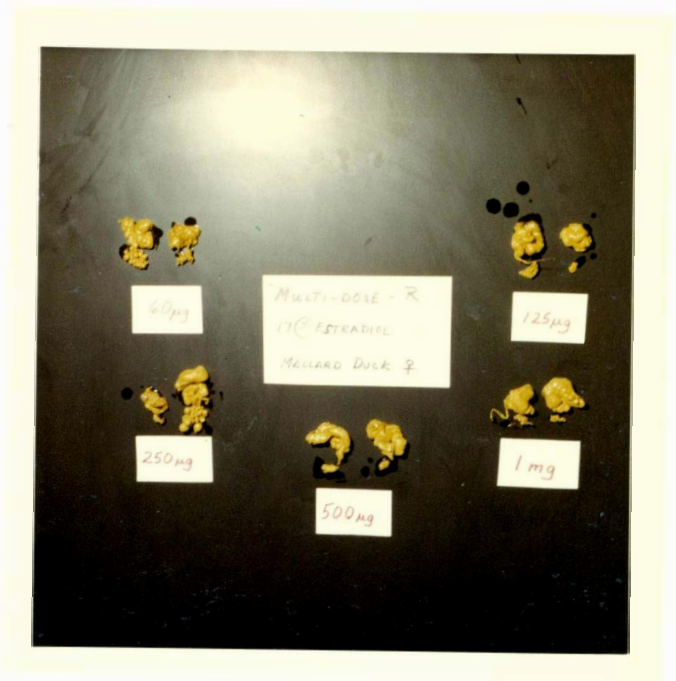

Figure 10: Ovaries and oviducts of ducks in the R-estrogen experiment.

In summary, both estrogen experiments demonstrated that estradiol treatment brings about changes in blood chemistry much more readily than it influences oviductual development at this time of year. At low dosage levels (60 micrograms of estradiol) calcium and A/G ratio changes appreciably in response to hormone treatment, whereas oviductual development was minimal. However, oviductual development may not be dependent on estrogen alone, but may be enhanced or inhibited by other factors present in the non-breeding mallard female.

LH Experiment

The results of the LH experiment are presented in Table 8. Mean experimental control levels of calcium, sodium, potassium and albumin were determined from ducks in group 1. As a comparison, control levels for the same blood constituents were tabulated for the farm control ducks from blood samples taken November 18 and 25, during the time of the LH experiment. Mean treatment values of serum calcium, sodium, potassium and albumin were obtained for each group receiving PLH or CPX from serum specimens taken after the first hormone treatment. Data, including pre- and post- treatment weights, and serum constituent levels on days when blood samples were taken, are presented for individual birds in Appendix F.

Farm control birds were found to have the same concentration of serum calcium as the experimental control birds. Serum

Table 8: Mean serum calcium, sodium, potassium, and albumin for ten farm control ducks, experimental control ducks and ducks receiving different dosages of Pituitary Luteinizing Hormone (PLH) or a chicken pituitary extract (CPX).

Group (pairs)	Treatment	Number of samples	Calcium (mg%)	Sodium (mg%)	Potassium+ (mg%)	Albumin (gm%)
farm control		20	10.4	328	19.5	3.7
1	control	16	10.4	326	16.6	3.3
2	0.5mg PLH	14	9.7	320	17.7	3.7
3	1.0mg PLH	14	9.5	325	17.7	3.3
4	2.5mg PLH	11	9.3*	328	17.7	3.0
5	2.5 CPX	14	10.1	328	19.9	3.1

* 2.5mg PLH calcium/experimental control calcium, $t = 2.76$, df = 25

+ Potassium statistical analysis, farm control/experimental control, $t = -7.82$ df = 34

sodium and albumin levels were not appreciably different (Table 8). However, potassium was significantly lower at the .01 level in experimental controls than in farm controls. Further study would be required to find the specific reason for this, but a stress factor possibly attributable to handling and confinement may have been involved.

All groups receiving PLH treatment showed serum calcium levels slightly lower than the experimental control birds. This decrease in calcium was shown to be greater as the dosage of PLH increased. In fact, the birds receiving 1.0 milligrams of PLH showed significantly lower serum calcium values, at the .10 level, than those encountered in the experimental control ducks. Since the estrogen experiments suggest that serum calcium levels are a fairly good index to estrogen concentration, the lower serum calcium levels found here may indicate a low estrogen titer (or lack of estrogen) also. Moreover, as Sturkie (1965) suggests, in the light of chicken research, estrogen secreted by the ovary probably cuts down the release of pituitary gonadotrophins, such as LH. If the reverse is also true; then, the high circulating levels of PLH might inhibit ovarian estrogen secretion. This suggests that in this experiment PLH may have interfered with the release of ovarian estrogen as measured by serum calcium levels.

Serum sodium, potassium and albumin in PLH treated ducks showed mean values not appreciably different from those found in the experimental control ducks (Table 8).

In the CPX treated ducks, serum calcium, sodium, and potassium differed only slightly from the values found in the control ducks. On the other hand, albumin showed a slight depression from the albumin values found in the control ducks.

It was the intent of this experiment to see if PLH or CPX had any effects on blood chemistry or ovarian and oviductual development. Few changes were noted that could be directly related to these hormones (Figure 11). Possible reasons why PLH or CPX might not demonstrate any blood chemical or morphological changes are numerous, but only those considered most salient will be mentioned here. In the first place, the non-breeding duck may be totally unresponsive to gonadotrophins of any source, though many researchers believe refraction terminates before the time of this study (mid-November) and that refractiveness is hypothalamic and not gonadal. Secondly, gonadotrophins from a source other than the duck may not function in the mallard; and thirdly, the dosage levels of the hormones used may not have been sufficient to ellicit any specific morphological or blood chemical change at this time.

S-CPX Experiment

In the S-CPX experiment, different dosages of chicken whole pituitary extract were injected in an attempt to stimulate ovarian follicular development and oviductual growth with some possibility of egg laying. Near the conclusion of the experiment, many ducks

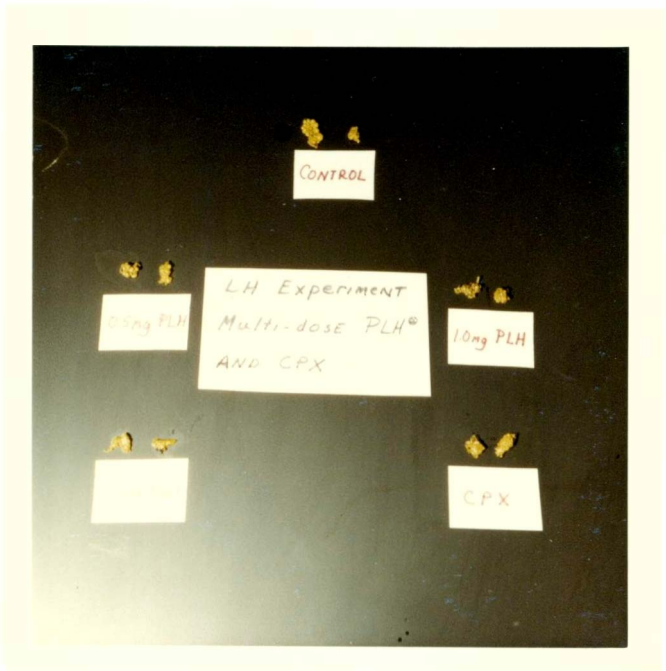

Figure 11: Ovaries of ducks receiving different daily dosages of Pituitary Luteinizing Hormone.

began egg-laying activity. Since control ducks as well as experimental ducks entered the laying state, the effect of the chicken pituitary extract could not be measured as previously anticipated. The higher temperature of the indoor environment was believed to have hastened egg laying, since the photoperiod used corresponded with the natural light cycle at that date. In spite of the change in plans, much was learned from the marked changes noticed in the blood chemical composition of many of the ducks, both control and experimental. In late January and early February, the farm control and reserve flocks also showed some signs of gonadal recrudescence (pairing and copulatory activity); however, the first egg was not laid until early March.

On day 1 of the experiment, ducks S-1, S-2, S-3, S-4, S-6, S-7, and S-10 had serum calcium values, total protein values and A/G ratios similar to those established earlier, as a part of this study, for the non-breeding mallard hen. The subsequent changes in these serum constituents are presented in Table 9 on days of the experimental period when the blood samples from which they were calculated were taken. From this table the following points are evident.

1. On the first day, blood samples from these birds showed values for serum calcium close to to the 10.8 milligrams percent established for the non-breeding mallard. The samples taken thereafter showed considerable increases in calcium values.

Table 9: Changes in serum calcium (Ca), total protein (TP) and albumin to globulin ratio (A/G) during transition from non-breeding to breeding state for seven female mallard ducks showing non-breeding levels of serum calcium on day one.

Duck		1	4	7	10	13	16	19	23 day
S-1	Ca	11.2	12.3	12.9	12.6	14.2	14.0	17.4	19.1
	TP	4.7			7.5				6.4
	A/G	2.6			2.0				.78
S-2	Ca	11.9	10.3	12.4	13.9	17.8	21.2	22.1	20.9
	TP	5.0			5.2				4.8
	A/G	1.7			1.2				.78
S-3	Ca	11.1	10.3	12.1	14.2	17.0	24.3	18.7	23.0
	TP	4.1			4.9				5.5
	A/G	1.7			1.1				.57
S-4	Ca	10.8	12.6	12.5	11.2	12.1	13.9	15.0	18.7
	TP	4.7			4.7				6.1
	A/G	1.8			1.1				.85
S-6	Ca	10.3	10.6	11.9	9.3	---	10.6	12.4	16.5
	TP	4.6			4.2				5.7
	A/G	2.0			1.5				.84
S-7	Ca	11.1	12.9	17.8	16.5	22.1	24.3	17.4	18.7
	TP	4.3			6.0				7.2
	A/G	2.0			.82				.53
S-10	Ca	10.6	10.3	12.1	11.6	15.0	14.4	18.7	27.0
	TP	3.8			4.6				4.7
	A/G	2.2			1.4				.52

2. Nearly all birds demonstrated an increase in total protein as the experiment progressed.

3. All birds showed a decrease in the A/G ratio which was previously established as resulting from an increase in the globulin fraction and a decrease in albumin.

Both the elevations of calcium and total protein, and the decrease in the A/G ratio were similar to those found in the estrogen experiments which were a part of this study. Moreover, Smoes (personal communication) presented similar results in his study of the female mallard passing from non-breeding to breeding condition.

Ducks S-5, S-8, and S-9 all showed serum calcium values higher than those found in the other birds on the first day of the experiment. Total protein and A/G ratio, on day 1 of the experiment, were close to the values established in this study for the non-breeding mallard hen. Both serum calcium and total protein elevated as the experiment progressed. As it did with the other birds in this study, the A/G ratio decreased, indicating a reversal of the concentration of the two protein fractions. Serum calcium, total protein, and A/G ratio are presented for the above birds on the days blood samples were obtained (Table (10).

A point of interest in this experiment was the relationships between the serum calcium elevation, total protein elevation, and the A/G reversal, as a possible index to reproductive state. It was observed that the first sign of change from the non-breeding to the breeding state was the elevation of calcium.

Table 10: Changes in serum calcium (Ca), total protein (TP) and albumin to globulin ratio (A/G) for three female mallard ducks who showed higher than non-breeding levels of serum calcium on day one.

Duck		1	4	7	10	13	16	19	23 day
S-5	Ca	15.0	14.4	17.4	16.5	23.9	20.4	18.2	17.8
	TP	4.5			6.6				5.5
	A/G	1.4			1.2				.67
S-8	Ca	16.5	18.7	17.7	15.7	18.7	24.3	21.7	31.0
	TP	4.8			5.8				6.9
	A/G	1.2			.76				.53
S-9	Ca	17.8	15.7	26.3	27.0	25.5	17.0	16.5	25.9
	TP	5.0			7.0				6.0
	A/G	1.5			.67				.54

Secondly, the increase in total protein coincided with a decrease in the A/G ratio. However, the A/G ratio was not observed to reverse (values below 1.0) until serum calcium increased to a value of approximately 15.0 milligrams percent or even higher in some ducks.

Of further interest was the time lapse between appreciable changes in blood chemistry and the laying of the first egg. Of the birds that had calcium values close to 11 milligrams percent on the first day of the experiment (S-1, S-2, S-3, S-4, S-6, S-7, and S-10), all except S-6 laid eggs near the end of the blood sampling sequence or shortly thereafter (Table 11). For example, S-7, whose serum calcium and total protein was the highest of this group of birds on day ten, laid an egg on day 22 of the experiment. S-2 laid on the 24th day of the experiment, and S-10 laid on the 28th day. The remaining birds, except for S-6, laid eggs after the 28th day. S-6 was laparotomized, and her ovaries and oviducts were judged to be stimulated but still not quite mature. In correlation with this, serum calcium levels in S-6 were just approaching those levels found in the laying birds (Tables 9 and 11).

Another bird, S-11, not of this group, showed serum calcium values on days 1, 4, 7, 10, 13, 16, 19, and 23 of 10.1, 10.3, 13.5, 15.0, 26.6, 20.5, 19.1, and 24.3 respectively, and laid her first egg on day 17. Considering bird S-11 and assuming serum calcium is an acceptable index of reproductive state, only

Table 11: Egg laying record for eleven female mallard ducks during transition from the non-laying to the laying state.

Duck	1 to 15	16	17	18	19	20	21	22	23	24	25	26	27	28	29 to 35 day
S-1															XX
S-2										X					XXX
S-3															XX
S-4															XX
S-5					X			X		X					XXX
S-6															
S-7								X	X						XX
S-8														X	XXXX
S-9		X		X											X
S-10														X	XXX
S-11			X					X							XX

X denotes one egg

a short period of time is required for morphological changes, which bring the duck into the reproductive state.

The other group of ducks, S-5, S-8, and S-9, which showed a considerably higher than normal level of serum calcium at the beginning of the experiment, also laid eggs. Of this group, S-9 (control duck) laid an egg on day 16. S-9 also showed the highest serum calcium and total protein on the first day of the experiment for this group. Ducks S-5 and S-8 laid on days 19 and 28, respectively (Table 11).

It is interesting to note that even though S-8 had calcium values higher than normal at the beginning of the study, 28 days were required before her first egg was laid. This was considerably longer than the time required by many of the birds who showed non-laying levels of calcium at the beginning of the study. However, S-8 showed an elevation of serum calcium from days 1 to 4, then a depression on days 7 and 10, followed by a gradual rise again. These serum calcium changes in S-8 suggest a temporary interruption of those physiological alterations leading to the laying state, thereby lengthening the period of time required to become reproductively functional.

Table 12 presents mean serum potassium values for each duck receiving CPX treatment and for the control ducks. It points out that prior to the onset of lay and during the laying state, potassium values decrease from the values established for the non-breeding mallard female. Decker (1965) and Smoes

Table 12: Mean serum potassium levels for eleven female mallard ducks during transition from the non-laying to the laying state and receiving a chicken pituitary extract (CPX).

Duck	Treatment	Potassium in mg%
S-1	1 CPX*	18.2
S-2	1 CPX	17.1
S-3	3 CPX	16.9
S-4	3 CPX	17.6
S-5	2 CPX	17.0
S-6	2 CPX	17.3
S-7	5 CPX	17.5
S-8	5 CPX	17.8
S-9	control	18.4
S-10	control	17.4
S-11	5 CPX	18.1

* 1 CPX equivalent to 1 whole chicken pituitary
n = 8

Table 13: Mean levels of serum calcium, sodium, potassium, albumin and total lipid for a female mallard duck receiving Follicle Stimulating Hormone for a period of ten days.

Duck	Calcium (mg%)	Sodium (mg%)	Potassium (mg%)	Albumin (gm%)	Lipid (mg%)
S-12	12.4	336	17.8	3.1	370

n = 7 for calcium, sodium, potassium and albumin
n = 2 for lipid

(personal communication) also found potassium to be lower in female mallards during the laying period. The specific reason for the depression of potassium near the onset and during the laying period is not known.

Many of the CPX treated ducks showed a substantial rise in total serum lipid throughout the experiment (Appendix G). This has also been known to occur in chickens at the onset of laying, and it is believed to be caused by higher circulating levels of estrogen. Moreover, Smoes (personal communication) found a much greater concentration of serum lipid in the laying mallard hen than was established for the non-breeding mallard hen in this study.

The remaining duck, S-12, utilized in this study, was treated with ovine FSH and this bird showed mean calcium, sodium, potassium and albumin values as they appear in Table 13. The raw data for these constituents appears in Appendix G. Blood chemical analysis and ovarian and oviductual development showed that S-12 was not stimulated by the FSH. When comparing S-12 with the other birds in this experiment, one might conjecture that ovine FSH inhibits follicular development, at least under the conditions existent in this study. However, additional research with mammalian FSH using mallard ducks is needed to substantiate this assumption.

Since nearly all of the ducks receiving CPX treatment laid eggs near the end or soon after the experiment was concluded,

their serum calcium changes and protein changes (potassium and lipid were less reliable) can be said to be fairly good indications of the natural reproductive state and not of response to CPX treatment.

SUMMARY

Weekly blood samples were taken from 10 non-breeding female mallard ducks for a period of four months, October through January. Normal levels of serum calcium, sodium, potassium, albumin, globulin and total lipid were established, and the effects of estrogenic and gonadotrophic hormone treatment on these blood chemical constituents in several other groups of non-breeding female mallards were measured. In addition, gross morphological changes in the reproductive organs were noted.

Two estrogen experiments were conducted using different daily dosages of estradiol. Serum specimens were collected from each treatment group both prior to and during the treatment period. In both estrogen experiments, an increase in serum calcium, significant at the .01 level, was noted for all treatment groups except those receiving 25 micrograms. Also, an increase in serum globulin and a decrease in serum albumin, both significant at the .01 level, were found for most treatment groups. In one of the experiments, a large elevation in serum lipid followed treatment.

Variations of serum potassium and sodium in both experiments could not be directly related to estradiol treatment. However, in one estrogen experiment and in one gonadotrophic experiment, differences, significant at the .05 level, were

found between experimental control levels and farm control levels of serum potassium. Though the specific reason for this difference was not known, a stress factor was believed to have been involved.

The results of the estrogen experiments showed an increasing responsiveness to estradiol treatment as the ducks came nearer the breeding period. Higher serum calcium levels and more developed oviducts were observed even though dosage levels were lower. In any case, estrogen treatment seemed to affect serum calcium levels much more readily than oviductual development.

In another experiment, ovine Pituitary Luteinizing Hormone (PLH) was administered daily. This treatment did not appear to have any direct effect on ovarian follicular or oviductual development, but it did result in a decrease in serum calcium levels.

Blood specimens showed dramatic changes in chemical components following treatment with chicken pituitary extract (CPX), but because of the onset of the laying state, these changes could not be directly related to hormone treatment. In this experiment, changes were particularly evident in serum calcium and protein levels as the ducks passed from the non-breeding to the laying condition. In fact, serum calcium showed a twofold increase in most birds laying eggs. Total protein also increased

appreciably, though most of the increase was found in the globulin fraction while albumin decreased. Potassium levels in the laying birds were lower than those found in the non-breeding ducks.

These changes in serum constituents, taking place with the transition from non-breeding to breeding state, also occurred with estrogen treatment. This suggests that the changes, in both cases, were probably primarily caused by estrogen. Furthermore, since serum calcium levels increase and A/G ratios decrease, both in connection with the laying state and estrogen treatment, they could possibly serve as indexes to reproductive state.

An attempt to stimulate ovarian follicular growth with equine Follicle Stimulating Hormone (FSH) yielded inconclusive results.

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APPENDIX A

Ingredients of
Napiana Duck Developer Pellet

Ingredients of Napiana Duck Developer Pellet

Ground Yellow Corn, Pulverized Oats, Meat and Bone Meal, Soybean Meal, Dehydrated Alfalfa Meal preserved with Ethoxyquin, Corn Gluten Feed, Wheat Middlings, Wheat Red Dog, Folic Acid, Cane Molasses, Vitamin A Palmitate, D-Activated Animal Sterol (Source of Vitamin K), Vitamin B12 Supplement, Riboflavin, Niacin, Calcium Pantothenate, Choline Chloride, Ground Limestone, Dicalcium Phosphate, Salt, Trace amounts of Magnesium Carbonate, Manganous Oxide, Iron Carbonate, Iron Oxide, Copper Oxide, Cobalt Carbonate, Zinc Oxide and Calcium Iodate.

Manufactured by

NAPPANEE MILLING CO., INC.

Nappanee, Indiana

APPENDIX B

Assay for
Follicle Stimulating Hormone

Assay for Follicle Stimulating Hormone, ovine, NIH-FSH-S3.*

Activity assayed	No. of assays	Mean Relative Potency	95% Limits	Standard Preparation	Method of Assay
FOLLICLE STIMULATING HORMONE	5	1.1	0.95-1.27	NIH-FSH-S1***	HCG augmentation
		CONTAMINATING ACTIVITIES			
Luteinizing Hormone	3	0.0044	0.003-0.0059	NIH-LH-S1**	Ovarian Ascorbic Acid Depletion
Thyroid Stimulating Hormone	2	0.0014	0.001-0.0019	U.S.P. Thyrotrophin	Thyroidal P^{32}
Growth Hormone	1	0.02**	--	U.S.P. Growth Hormone	Body wt. gain
Prolactin	1	0.20**	--	House Standard+ (27.0 IU/mg)	Pigeon crop sac
Other activities		NOT ASSAYED			

* from Pituitary Hormone Distribution Program, National Institutes of Health, Bethesda, Maryland.
** Mean relative potency expressed as units of activity per mg.
*** Activity defined as 1 unit/mg
+ Calibrated against International standard

APPENDIX C

Tables of Raw Data for the Farm Control Ducks

Duck no.		10/14	10/21	10/28	11/4	11/11	11/18	11/25	12/2	12/9	12/16	12/23	12/30	1/6	1/13	1/20	1/27	Mo./day
2	Ca	12.3	11.5	11.3	11.3	11.3	10.5	10.8	10.3	10.2	9.0	--	10.6	11.0	11.1	11.4	10.8	mg%
	Na	289	304	340	340	340	348	322	313	322	322	420	322	342	348	358	332	mg%
	K	18.1	16.6	17.7	17.4	19.6	19.6	20.4	19.9	19.9	18.5	21.8	18.9	20.9	22.0	21.1	17.8	mg%
3	Ca	10.6	11.1	10.7	9.3	10.9	9.8	10.2	8.4	9.6	9.5	13.2	9.9	9.9	10.2	10.9	10.8	
	Na	304	322	322	322	332	322	322	278	340	322	410	313	332	332	340	340	
	K	17.7	17.7	17.0	18.1	18.1	18.5	20.4	17.4	19.5	18.6	--	17.4	21.3	18.5	23.4	18.1	
4	Ca	13.9	10.7	10.6	11.2	10.9	8.9	9.8	10.3	9.3	10.3	11.2	9.8	9.6	10.9	10.7	11.4	
	Na	322	313	322	358	332	332	313	313	346	332	322	313	322	348	358	348	
	K	20.4	17.0	19.9	20.9	19.6	19.9	19.2	20.4	20.0	19.6	20.5	19.2	21.1	18.5	19.7	18.1	
5	Ca	11.1	11.2	--	10.1	10.9	10.3	10.3	9.7	9.0	11.5	10.1	--	9.9	9.2	11.6	12.4	
	Na	332	332	--	348	340	348	332	289	332	322	332	304	322	340	366	358	
	K	18.1	18.5	--	17.7	19.6	18.1	18.9	25.6	18.8	18.9	17.7	17.7	18.6	21.9	19.3	18.1	
6	Ca	10.6	11.6	10.0	9.6	9.2	9.9	10.2	9.5	9.6	--	12.0	11.3	10.3	10.4	10.7	10.8	
	Na	322	332	332	313	322	340	313	296	332	--	348	304	312	340	340	332	
	K	17.7	18.1	17.4	17.3	19.6	18.1	17.7	17.4	19.2	--	16.7	16.6	20.1	20.6	19.5	19.9	
8	Ca	10.9	12.1	12.0	10.5	10.8	10.7	10.6	12.0	10.5	10.1	10.9	11.6	9.3	10.3	10.8	11.6	
	Na	332	322	322	332	--	348	322	332	--	332	376	322	312	340	332	376	
	K	16.6	16.6	19.6	18.5	--	22.7	19.2	21.1	20.8	21.1	21.1	17.7	24.5	22.9	18.6	17.2	
9	Ca	10.6	13.3	11.5	10.5	11.3	10.8	11.3	9.9	11.2	10.3	12.0	11.3	9.8	10.3	11.1	10.5	
	Na	304	358	332	322	340	340	322	313	332	340	384	332	312	332	340	348	
	K	16.6	19.2	20.4	19.9	22.6	20.9	19.2	19.6	20.0	20.3	21.1	18.5	24.5	21.3	19.0	18.7	
10	Ca	11.9	11.6	10.3	10.8	11.0	10.3	11.3	10.2	11.1	9.7	11.6	11.9	10.8	11.5	11.2	11.9	
	Na	313	322	304	332	332	322	304	313	340	322	366	332	295	313	332	348	
	K	18.5	18.1	18.1	19.9	18.9	19.9	18.9	18.9	21.9	18.5	19.9	18.5	17.0	19.0	19.3	17.0	

Weekly variation in serum calcium (Ca), sodium (Na), potassium (K) for farm control ducks.

Duck no.		10/21	10/28	11/4	11/11	11/18	11/25	12/2	12/9	12/16	12/23	12/30	1/6	1/13	1/20	1/27	Mo. day
2	ALB	2.94	2.94	3.32	2.75	3.52	--	--	3.39	3.32	2.29	3.14	--	3.14	3.58	3.58	gm%
	LIP				200				487					200			mg%
3	ALB	--	3.03	5.56	2.88	3.52	3.78	--	3.58	3.52	2.88	3.20	2.94	2.69	3.90	3.71	
	LIP					380			500					240			
4	ALB	3.90	3.90	3.71	2.94	3.58	3.71	--	2.50	3.32	2.88	3.32	3.52	2.88	3.78	3.71	
	LIP				450				143					100			
5	ALB	4.60	--	3.03	3.03	3.20	3.39	--	2.62	2.88	2.69	2.75	3.20	5.44	3.32	2.94	
	LIP				450				180					440			
6	ALB	3.32	--	4.22	4.22	3.39	3.58	--	3.39	4.47	2.75	3.14	3.32	3.39	3.14	3.14	
	LIP				240				225					180			
8	ALB	3.52	4.73	4.71	--	--	3.90	--	3.58	3.52	3.71	3.03	5.44	3.39	3.14	3.20	
	LIP				533				300					240			
9	ALB	3.20	--	3.90	3.20	--	--	3.60	3.52	4.06	3.58	2.75	4.73	3.14	3.14	3.78	
	LIP								200					630			
10	ALB	3.20	--	3.14	3.20	2.75	3.71	5.00	--	2.43	2.75	2.75	3.39	2.75	3.90	4.73	
	LIP				340				380					167			

Weekly variations in serum albumin (ALB) and total lipid (LIP) for farm control ducks.

Changes in total serum protein (TP), albumin (ALB) and globulin (GLB) for the farm control ducks.

Duck no.		Oct. 14	Nov. 18	Dec. 30	Jan. 27	
1	TP	--	5.1	4.6	4.0	gm%
	ALB	--	3.2	3.2	2.7	gm%
	GLB	--	1.9	1.4	1.3	gm%
2	TP	3.8	4.8	4.6	4.0	
	ALB	2.4	3.6	3.7	2.7	
	GLB	1.4	1.2	0.9	1.3	
3	TP	4.2	4.8	4.1	4.7	
	ALB	2.6	3.7	3.1	2.8	
	GLB	1.6	1.1	1.0	1.9	
4	TP	4.5	4.3	4.4	4.4	
	ALB	2.3	2.4	2.9	1.5	
	GLB	2.2	1.9	1.5	2.0	
5	TP	5.5	4.4	4.8	4.2	
	ALB	3.5	2.7	3.8	2.8	
	GLB	2.0	1.7	1.0	1.4	
6	TP	6.0	4.7	4.7	4.1	
	ALB	3.7	3.0	3.6	2.7	
	GLB	2.3	1.7	1.1	1.4	
7	TP	6.1	5.0	4.5	4.6	
	ALB	3.8	2.9	3.0	3.0	
	GLB	2.3	2.1	1.5	1.6	
8	TP	6.1	5.9	5.2	4.6	
	ALB	3.8	3.9	3.9	3.2	
	GLB	2.3	2.0	1.3	1.4	
9	TP	5.5	5.2	5.0	4.4	
	ALB	3.4	2.9	3.7	2.8	
	GLB	2.1	2.3	1.3	1.6	
10	TP	4.8	4.9	5.0	4.5	
	ALB	2.9	3.0	4.0	2.9	
	GLB	1.9	1.9	1.0	1.6	

APPENDIX D

Tables of Raw Data For the Ducks of the E-estrogen Experiment

Pre-treatment and Post-treatment weights of the ducks in the E-Estrogen experiment.

Duck No.	Group	Pretreatment wt. (gms)	Post-treatment wt. (gms)
1	1	1250	889
2	1	1264	1100
3	2	1238	983
4	2	1370	992
5	3	1304	1132
6	3	1342	990
7	4	1379	1018
8	4	1212	1011

Changes in serum calcium (Ca), sodium (Na), potassium (K), albumin (ALB) and lipid (LIP) for the ducks in the E-Estrogen Experiment.

Duck no.		Hormone treatment period: -3	1	3	5	8	11	14	day
1	Ca	10.3	10.6	9.9	9.0	10.1	10.3	10.8	mg%
	Na	322	322	332	366	348	332	340	mg%
	K	17.0	16.7	19.7	19.7	19.9	18.1	19.7	mg%
	ALB	3.7	4.2	4.2	3.8	2.8	3.1	3.4	gm%
	LIP	666							mg%
2	Ca	12.1	10.8	9.7	10.5	9.6	13.3	12.7	
	Na	348	353	332	348	278	327	332	
	K	17.4	19.7	16.7	22.2	18.1	17.9	18.6	
	ALB	3.9	3.8	3.6	4.6	4.1	3.1	3.2	
	LIP	140							
3	Ca	9.5	8.3	10.9	14.8	19.1	16.5	21.7	
	Na	317	313	332	304	332	332	318	
	K	17.0	18.8	17.0	17.4	21.3	17.4	17.2	
	ALB	4.1	4.9	3.3	3.6	4.1	3.5	4.1	
	LIP	134							
4	Ca	10.6	9.9	11.2	14.8	16.1	16.1	17.0	
	Na	340	322	340	322	340	318	--	
	K	17.2	18.6	17.9	19.0	18.6	18.6	22.7	
	ALB	3.7	4.6	3.6	3.1	4.5	3.0	4.2	
	LIP	100							

Duck no.		-3	Hormone treatment period 1	3	5	8	11	14	day
5	Ca	10.2	9.8	16.5	32.0*	24.7	20.4	23.0	mg%
	Na	322	300	340	384	304	308	--	mg%
	K	16.3	17.3	24.5	24.3	19.3	18.8	20.9	mg%
	ALB	3.3	4.7	5.6	6.4	5.6	5.7	5.9	gm%
	LIP	333					2500		mg%
6	Ca	11.6	10.6	12.7	26.3	31.0*	27.0*	19.1	
	Na	362	300	340	322	269	318	--	
	K	19.5	19.5	19.3	18.5	17.0	19.7	18.6	
	ALB	4.0	3.9	4.8	4.5	5.5	4.7	4.6	
	LIP	280							
7	Ca	11.1	10.5	15.0	26.3	21.3	39.0*	25.5	
	Na	322	304	348	304	261	269	--	
	K	16.0	18.1	19.9	19.7	16.7	15.5	15.8	
	ALB	4.1	4.4	3.5	5.7	5.7	4.8	4.1	
	LIP	425					3700		
8	Ca	10.5	9.9	19.1	23.0	35.0*	27.0*	30.0*	
	Na	313	289	340	358	278	292	322	
	K	16.7	16.5	19.5	20.6	16.0	17.9	19.9	
	ALB	3.3	5.0	3.6	6.5	5.8	4.2	5.3	
	LIP	130							

* estimated

Changes in serum total protein (TP), albumin (ALB) and globulin (GLB) for ducks of group 2 and 3.

Duck no.	Group	Three days prior to treat. TP (gm%)	ALB (gm%)	GLB (gm%)	Fifth day of treatment. TP (gm%)	ALB (gm%)	GLB (gm%)
3	2	5.2	3.4	1.8	6.4	3.2	3.2
4	2	5.7	3.6	2.1	5.0	2.8	2.2
5	3	4.8	2.6	2.2	7.8	2.5	5.3
6	3	4.8	2.8	2.0	7.5	2.7	4.8

APPENDIX E

Tables of Raw Data For the Ducks of the R-estrogen Experiments

Pre-treatment and Post-treatment weights of the ducks in the R-Estrogen experiment.

Duck no.	Group	Pretreatment wt. (gms)	Post-treatment wt. (gms)
1	1	1062	956
2	1	1209	1023
3	2	1174	1036
4	2	1337	1185
5	3	1153	954
6	3	1364	1193
7	4	1550	1238
8	4	1224	1096
9	5	1236	1075
10	5	1246	1093

Changes in serum calcium (Ca), sodium (Na), potassium (K), albumin (ALB) and lipid (LIP) for the ducks in the R-Estrogen Experiment.

Duck no.		Hormone treatment period							
		-3	1	3	7	10	13	15	day
1	Ca	11.1	10.5	18.1	16.1	19.1	20.9	19.1	mg%
	Na	332	353	376	322	340	332	332	mg%
	K	16.3	24.5	17.0	18.5	21.1	17.7	17.2	mg%
	ALB	3.3	3.2	3.3	3.9	5.0	4.4	2.4	gm%
	LIP	220						580	mg%
2	Ca	11.8	16.5	22.5	17.8	21.3	26.3	12.2	
	Na	348	358	366	313	332	376	250	
	K	17.0	21.3	15.8	18.1	20.9	21.1	16.3	
	ALB	2.7	3.5	3.4	3.7	4.1	3.3	3.4	
	LIP	640						540	
3	Ca	11.3	11.5	24.2	32.5*	30.0*	31.5*	25.5	
	Na	348	353	358	332	313	358	340	
	K	17.4	20.9	14.9	19.9	18.9	17.7	16.3	
	ALB	2.6	3.5	4.1	4.9	5.4	4.7	3.5	
	LIP							420	

Duck no.		Hormone treatment period							
		-3	1	3	7	10	13	15	day
4	Ca	11.2	11.8	22.3	28.4*	29.0*	32.4*	26.7	mg%
	Na	353	348	322	332	322	322	322	mg%
	K	21.3	22.7	16.7	19.6	22.5	17.7	17.2	mg%
	ALB	3.0	2.8	3.7	5.2	5.2	4.4	2.7	gm%
	LIP	640						633	mg%
5	Ca	19.9	21.1	23.8	23.4	22.5	--	14.8	
	Na	353	340	406	340	332	--	289	
	K	17.2	19.7	19.5	19.6	19.6	--	20.6	
	ALB	4.1	3.4	3.9	5.2	5.3	5.6	3.6	
	LIP	620						825	
6	Ca	10.6	12.2	29.0*	26.7	30.4*	32.6*	22.1	
	Na	340	340	348	358	313	322	322	
	K	18.6	18.1	19.3	20.4	18.5	18.5	13.5	
	ALB	3.6	4.2	4.7	5.3	4.9	5.4	3.1	
	LIP	1160						625	
7	Ca	10.8	10.6	29.4*	36.6*	42.0*	39.0*	30.0*	
	Na	344	340	340	340	313	322	313	
	K	16.3	17.2	19.7	20.9	20.4	18.5	17.9	
	ALB	3.3	3.6	4.7	4.7	5.7	5.7	4.6	
	LIP							840	
8	Ca	13.5	13.5	24.5	33.0*	31.2*	27.8*	17.8	
	Na	362	358	340	322	322	322	278	
	K	17.2	17.4	16.0	19.2	19.9	19.2	23.1	
	ALB	3.3	3.6	3.3	5.3	5.0	5.0	5.9	
	LIP							440	
9	Ca	11.6	11.1	14.3	40.0*	40.0*	40.0*	36.0*	
	Na	332	362	332	296	313	332	278	
	K	15.1	17.9	17.4	19.2	19.2	18.5	29.0*	
	ALB	2.8	3.6	5.0	6.1	6.6	5.6	5.6	
	LIP	450						560	
10	Ca	10.5	10.6	28.1*	41.0*	44.0*	40.0*	30.9*	
	Na	332	358	348	332	348	332	289	
	K	15.8	19.4	22.9	21.9	21.5	23.4	27.1	
	ALB	2.5	2.9	5.3	5.4	5.9	5.6	4.5	
	LIP	540						600	

*estimated

Changes in total protein (TP), albumin (ALB) and globulin (GLB) for the ducks in the R-Estrogen experiment.

Duck no.		-3	1	10	15	days
1	TP	4.3	6.7	8.8	5.5	gm%
	ALB	2.6	4.1	2.7	1.9	gm%
	GLB	1.7	2.6	6.1	3.6	gm%
2	TP	4.7	5.5	7.8	3.5	
	ALB	3.2	3.5	2.7	1.3	
	GLB	1.5	2.0	5.1	2.2	
3	TP	5.2	5.2	7.8	3.8	
	ALB	3.2	3.3	2.5	1.3	
	GLB	2.0	1.9	5.3	2.5	
4	TP	4.3	4.8	8.7	5.6	
	ALB	2.0	3.2	2.6	1.9	
	GLB	2.3	1.6	6.1	3.7	
5	TP	5.6	5.4	7.3	4.0	
	ALB	3.2	3.6	3.1	1.6	
	GLB	2.4	1.8	4.2	2.4	
6	TP	5.9	6.2	8.6	5.5	
	ALB	3.0	2.9	3.0	2.0	
	GLB	2.9	3.3	5.6	3.5	
7	TP	9.4	5.3	8.6	4.1	
	ALB	5.3	3.0	3.5	1.0	
	GLB	4.1	2.3	5.1	3.1	
8	TP	5.4	5.6	9.8	5.8	
	ALB	3.5	3.6	2.7	1.9	
	GLB	1.9	2.0	7.1	3.9	
9	TP	5.0	5.4	10.4	5.1	
	ALB	3.2	3.2	4.4	1.5	
	GLB	1.8	2.2	6.0	3.6	
10	TP	4.5	3.8	8.8	4.2	
	ALB	3.0	2.4	2.7	1.3	
	GLB	1.5	1.4	6.1	2.9	

APPENDIX F

Tables of Raw Data
For the Ducks of the LH Experiment

Pre-experimental and post-experimental weights for the ducks in LH-Experiment.

Duck no.	Group	Pre-experimental wt. (gms)	Post-experimental wt. (gms)
1	1	1049	990
2	1	1085	1000
3	2	1019	982
4	2	1048	962
5	3	1178	1043
6	3	940	853
7	4	1000	923
8	4	1153	1020
9	5	982	896
10	5	961	911

Changes in serum calcium (Ca), sodium (Na), potassium (K), albumin (ALB) and lipid (LIP) for the ducks in the LH-Experiment.

Duck no.		Hormone treatment period: -2	2	4	6	8	10	+2	+4 day	
1	Ca	10.9	8.6	9.5	8.9	11.2	9.8	10.9	9.8	mg%
	Na	348	322	313	340	340	322	332	296	mg%
	K	16.5	15.9	16.3	18.1	17.5	16.3	17.4	14.2	mg%
	ALB	3.5	3.1	3.6	4.6	3.0	3.0	3.1	--	gm%
	LIP	800							620	mg%
2	Ca	11.2	11.1	10.9	11.1	10.2	10.5	11.1	10.8	
	Na	340	322	322	322	322	322	322	332	
	K	15.1	16.3	16.4	17.4	16.6	17.7	17.2	16.5	
	ALB	3.2	3.1	3.4	3.4	3.1	3.5	3.1	3.2	
	LIP	650							480	
3	Ca	10.1	9.6	9.6	--	9.0	9.0	10.3	9.9	
	Na	366	340	304	--	322	313	313	322	
	K	15.5	21.9	15.9	--	16.3	17.0	17.7	17.3	
	ALB	4.4	--	3.9	4.6	2.9	3.2	3.6	3.3	
	LIP									

Duck no.		-2	Hormone treatment period 2	4	6	8	10	+2	+4 day
4	Ca	11.2	10.3	9.6	9.5	9.3	9.6	10.6	9.9
	Na	348	332	304	322	332	332	313	322
	K	14.7	17.4	15.9	16.2	17.3	17.0	20.6	19.7
	ALB	3.5	--	3.6	5.0	3.0	3.3	4.4	3.6
	LIP	520							580
5	Ca	9.5	10.1	9.8	8.0	9.3	8.8	11.6	10.4
	Na	340	322	322	313	332	322	332	340
	K	16.0	17.0	16.3	17.4	17.0	18.1	20.1	14.9
	ALB	4.5	3.4	3.1	4.7	3.0	3.6	2.9	3.3
	LIP	540							720
6	Ca	10.6	10.1	8.8	7.6	8.8	9.0	11.6	9.2
	Na	340	313	313	332	322	313	322	358
	K	16.0	17.7	16.6	17.0	17.0	19.6	19.7	19.3
	ALB	3.4	3.2	3.0	3.5	2.7	3.1	2.9	3.5
	LIP	825							580
7	Ca	10.2	10.1	9.8	--	8.6	9.8	11.2	8.6
	Na	332	332	313	--	322	322	348	322
	K	14.5	17.0	17.0	--	17.0	18.9	16.5	17.2
	ALB	3.3	3.0	3.0	--	3.2	3.2	3.0	3.2
	LIP	780							520
8	Ca	10.1	9.2	9.5	7.2	10.2	8.5	--	--
	Na	340	322	332	332	340	322	--	--
	K	18.1	17.0	17.7	17.7	18.9	19.9	--	--
	ALB	--	--	3.1	2.8	2.9	3.0	--	--
	LIP	--							--
9	Ca	10.8	10.7	10.6	10.9	10.2	8.2	9.2	9.9
	Na	340	322	322	332	340	304	304	358
	K	15.3	16.3	18.1	19.2	18.8	21.5	25.8	19.9
	ALB	3.7	3.3	3.4	2.6	2.8	2.8	3.4	3.4
	LIP	680							560
10	Ca	10.2	9.9	--	9.8	10.1	10.9	11.2	9.8
	Na	322	332	--	322	332	313	340	348
	K	16.5	18.9	17.7	19.6	19.2	20.9	22.3	--
	ALB	3.6	--	3.9	3.5	3.0	2.7	3.1	3.0
	LIP	--							680

APPENDIX G

Tables of Raw Data
For the Ducks of the S-CPX Experiment

Pre-experimental and post-experimental weights of the ducks in the S-CPX Experiment.

Duck no.	Pre-experimental wt. (gms)	Post-experimental wt. (gms)
S-1	1250	1130
S-2	1232	1143
S-3	1395	1285
S-4	1376	1214
S-5	1100	990
S-6	1118	996
S-7	1280	1115
S-8	1282	1267
S-9	1189	1145
S-10	1677	1334
S-11	842	783
S-12	1005	987

Changes in serum sodium (Na), potassium (K), albumin (ALB) and lipid (LIP) for the ducks in the S-CPX Experiment.

Duck no.		1	4	7	10	13	16	19	23 day	
S-1	Na	322	366	332	340	340	322	366	358	mg%
	K	17.0	18.9	18.1	17.4	19.9	15.9	19.2	19.2	mg%
	ALB	--	4.1	3.8	4.4	4.9	3.7	--	3.9	gm%
	LIP		220				560			mg%
S-2	Na	313	322	322	322	332	332	332	348	
	K	17.0	16.6	17.7	17.0	17.4	15.9	18.1	17.0	
	ALB	--	3.5	3.3	3.5	3.0	3.2	--	3.5	
	LIP		155				640			
S-3	Na	313	332	322	322	322	340	332	348	
	K	17.4	16.6	16.3	17.0	17.0	15.5	17.0	18.5	
	ALB	--	3.0	2.7	3.0	2.9	3.2	--	3.2	
	LIP		180				600			
S-4	Na	322	340	332	313	313	348	358	340	
	K	17.4	17.0	18.1	17.0	17.4	16.6	18.1	19.2	
	ALB	--	3.2	3.1	3.2	3.0	3.1	--	3.9	
	LIP		150					360		

Duck no.		1	4	7	10	13	16	19	23 day
S-5	Na	313	313	332	340	332	332	340	313
	K	17.5	15.8	18.6	16.6	17.7	16.3	18.1	17.7
	ALB	---	3.5	3.7	3.9	3.5	3.4	--	2.9
	LIP		180				820		
S-6	Na	304	313	348	313	--	313	332	332
	K	17.0	16.6	19.2	16.8	--	15.5	17.0	19.6
	ALB	--	3.1	3.3	3.1	2.6	--	2.7	3.7
	LIP		300				360		
S-7	Na	322	322	332	304	--	322	332	332
	K	17.7	17.4	17.3	17.7	18.9	15.9	17.7	17.4
	ALB	--	3.7	3.3	5.2	4.1	3.4	3.0	2.8
	LIP		180					680	
S-8	Na	322	353	338	332	322	340	340	340
	K	18.9	17.7	18.5	16.0	18.5	15.5	18.9	18.1
	ALB	--	3.3	3.7	3.6	3.1	3.7	3.6	4.1
	LIP		400				820		
S-9	Na	322	340	332	332	304	313	332	340
	K	20.2	17.7	18.5	18.1	18.1	17.0	18.5	18.8
	ALB	---	3.1	5.3	5.4	3.7	2.9	4.1	3.7
	LIP		300					300	
S-10	Na	322	313	332	322	289	322	340	353
	K	18.1	16.6	18.1	16.6	17.7	15.5	18.9	18.1
	ALB	--	3.0	3.9	3.4	2.9	2.9	2.9	3.7
	LIP		333				520		
S-11	Na	313	313	322	322	332	322	348	340
	K	19.2	16.3	18.3	17.6	17.4	17.0	19.2	18.9
	ALB	--	3.4	3.4	4.2	4.1	3.0	3.5	3.9
	LIP		265					780	
S-12	CaK	--	13.5	13.2	12.6	13.9	10.3	11.2	12.2
	Na	--	322	322	340	348	340	340	340
	K	--	17.4	19.2	18.9	18.9	14.8	18.5	18.1
	ALB	--	2.0	3.8	3.8	2.6	2.9	3.3	2.9
	LIP		230				480		

Ca = milligrams calcium in mg%

APPENDIX H

Table Summary of Statistical Analysis for Hormone Experiments

E-Estrogen experiment

Dosage	Pretreatment calcium (mg%)			/	Treatment calcium (mg%)			t	df
	$\bar{X}$	n	s	/	$\bar{X}$	n	s		
25ug	10.9	4	.79		10.6	10	1.37	.612	12
250ug	9.6	4	.96		15.7	9	3.43	-4.987	11
2.5mg	10.5	4	.77		23.8	10	6.18	-6.379	12
5.0mg	10.4	4	.52		26.1	10	7.16	-6.896	12

Grand mean pretreatment Potassium (m g%)			/	farm control potassium (mg%)			t	df
$\bar{X}$	n	s		$\bar{X}$	n	s		
17.63	16	1.22		19.85	20	1.77	-4.41	34

R-Estrogen experiment

Dosage (ug)	Pretreatment calcium (mg%)			/	Treatment calcium (mg%)			t	df
	$\bar{X}$	n	s	/	$\bar{X}$	n	s		
60	12.5	4	2.74		19.3	10	3.80	-3.776	12
125	11.4	4	.26		28.2	10	3.45	-15.182	12
250	14.6	3	5.56		25.1	9	5.37	-2.803	10
500	12.1	4	1.61		31.1	10	7.11	-7.960	12
1000	10.9	4	.50		35.4	10	8.87	-8.682	12

Pretreatment potassium			/	Treatment potassium (mg%)			t	df
$\bar{X}$	n	s	/	$\bar{X}$	n	s		
18.56	20	2.47		19.37	49	2.77	-1.188	67

Pretreatment albumin (gm%)			/	Treatment albumin (gm%)			t	df
$\bar{X}$	n	s		$\bar{X}$	n	s		
3.25	20	.66		2.17	20	.87	4.175	38

Pretreatment globulin (gm%)			/	Treatment globulin (gm%)			t	df
$\bar{X}$	n	s		$\bar{X}$	n	s		
2.16	20	.66		4.21	20	1.50	-5.209	38

LH-Experiment

Pretreatment Potassium (mg%)			/ Farm control potassium (mg%)			t	df
$\bar{X}$	n	s	$\bar{X}$	n	s		
16.58	16	.99	19.55	20	1.28	-7.818	34

Experimental control calcium (mg%)			/ Calcium for 2.5mg PLH (mg%)			t	df
$\bar{X}$	n	s	$\bar{X}$	n	s		
10.4	16	.84	9.3	11	1.07	2.758	25